95. The method of claim 83, wherein the reagent is a monoclonal antibody.

96. The method of claim 95, wherein the monoclonal antibody is 260.4.

97. The method of claim 83, wherein $CD34^-$ $CD38^-$ cells are isolated from the tissue.

98. The method of claim 97, further comprising isolating the long-term repopulating HSCs from other $CD34^-$ $CD38^-$ cells using an antibody which specifically binds one of a late marker other than CD38 and an early marker other than CD34.

99. The method of claim 83, wherein $CD34^-$ cells that do not express a late marker are isolated from the tissue.

100. The method of claim 99, wherein the $CD34^-$ cells do not express any late marker of the group consisting of CD2, CD3, CD4, CD7, CD8, CD15, CD16, CD19, CD20, CD33, CD38, CD45, CD56, CD71, and glycophorin A. --

REMARKS

Claims 1-5, 7-11, 18-32, 51-53, 69, and 71-100 are pending after entry of this Amendment. Claims 1, 7, 18, 23-26, 51, and 69 have been amended. Claims 6 and 39-44 have been canceled. Claims 76-100 have been added. Claims 1, 18, 51, 69, 80, and 83 are the only independent claims. The amendments and additions do not include new matter, as set forth in the ensuing section. No additional claim fee is believed to be due.

Support in the Specification

Three specification paragraphs were amended to delete reference to "pre-embryonic tissue." As the Examiner noted in the Office Action dated 30 January 2001, there is

no such tissue. Because the skilled artisan would recognize this, removal of the term does not add new matter.

Claim 1 was amended by incorporating the recitation of claim 6 therein. The recitation of claim 6 was also incorporated into the other independent claims - claims 18, 51, and 69.

Claims 7 and 26 were amended merely to alter their dependency.

Claim 18 was amended to recite that HPCs that do not express KDR on their surface are designated "KPR^- HPCs." This is merely standard terminology in the art, and would be recognized as such by the skilled artisan. Claim 18 was also amended to recite that KPR^+ HPCs are enriched for long-term repopulating HSCs, as disclosed in originally filed claims 1 and 18 and in the specification at page 15, lines 1-10. As suggested by the Examiner during the telephone interview, the wording of claim 18 was rearranged to clarify that there are two separations that are recited - separation of HPCs from the hematopoietic tissue and separation of KDR^+ HPCs from KDR^- HPCs.

Claim 23 was amended simply to simplify its language without altering its meaning.

Claim 24 was amended simply to separate the specific early markers previously recited therein into new claim 76.

Claim 25 was amended in two ways. As suggested by the Examiner during the telephone interview, the claim was amended to clarify that long-term repopulating HSCs are isolated from other HPCs, as is evident from claim 18, from which claim 25 depends. The claim was also amended to specify that long-term repopulating HSCs are isolated using an antibody specific for either an early marker or a late marker. This recitation is supported in the specification, for example, at page 5, lines 13-18.

New claim 80 simply combines the recitations of pending claims 1 and 23, and represents merely an alternative way of claiming the subject matter disclosed in the application.

New claims 81-100 were not discussed with the Examiner during the telephone interview, but are believed to be allowable.

New claims 77-79, 81, and 82 simply recite early and late markers disclosed in the specification, for example at page 5, lines 19-22, and at page 44, lines 11-13.

New claim 83 substantially incorporates canceled claim 39, and is written in independent form. New claims 84-100 depend from new claim 83 and substantially repeat recitations that are made in other pending and canceled claims.

For the reasons set forth above, the Applicants respectfully contend that the amendments and additions made herein do not include new matter.

Sequence Listing

The Examiner requested that a sequence listing be filed in compliance with 37 C.F.R. § 1.821-1.825. An appropriate sequence listing is enclosed, in paper and electronic formats and with an accompanying Statement to Support Filing.

Rejection of Claims Pursuant to 35 U.S.C. § 112, First Paragraph

Claims 23-27 and 39-44 stand rejected pursuant to 35 U.S.C. § 112, first paragraph.

Claims 25 and 40-44 stand rejected because, in the Examiner's view, the terms "lin^-" and "lin^+" are indefinite, and the specification has not enabled one to separate lin^- and lin^+ cells. The Applicants have discontinued use of these terms, and has in place thereof recited early and late markers, which are understood in the art and described in the specification (e.g., at page 22, lines 11-23). The Applicants respectfully contend that these amendments render the Examiner's rejection of claims 25 and 40-44 moot. The Applicants believe that the Examiner agreed during the telephone interview that recitation of early and late markers in place of lin markers rendered the previous rejection moot.

Claims 23-27, 39, and 40 stand rejected because the Examiner contends that the antibodies recited in these claims do not bind with KDR, and that the claimed methods are therefore not enabled. The Examiner correctly notes that the antibodies recited in these claims do not bind specifically with KDR. However, each of these rejected claims depends from claim 18, which recites use of a reagent that specifically binds with KDR. Thus, each of claims 23-27,

39, and 40 merely recites an additional HPC selection criterion, and it is irrelevant whether the additional antibodies recited in claims 23-27, 39, and 40 bind with KDR. The Examiner should keep in mind that it is "long-term repopulating HSCs" that are being prepared, not merely KDR^+ HPCs. As disclosed in the specification, for example at page 22, lines 11-13, long-term repopulating HSCs can be selected on the basis that the HSCs are KDR^+, display an early marker, and do not display a late marker. Therefore, the specification supports the assertion that long-term repopulating HSCs can be selected based on their expression of early markers and non-expression of late markers, in addition to their expression of KDR. The methods recited in claim 23-27, 39, and 40 are therefore sufficiently enabled. The Applicants believe that the substance of this paragraph was discussed with the Examiner during the telephone interview, and that the Examiner agreed that, as amended, the claims clearly recited methods that are enabled by the specification.

For the foregoing reasons, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 23-27 and 39-44 pursuant to 35 U.S.C. § 112, first paragraph.

Rejection of Claims Pursuant to 35 U.S.C. 112, Second Paragraph

The Examiner rejects claims 18-32, 39-44, and 75 pursuant to 35 U.S.C. § 112, second paragraph. In the Examiner's view, independent claim 18 (from which the other rejected claims depend) is confusing with regard to whether HPCs or HSCs are being isolated and as to whether they are being isolated in a single step or in two discrete steps.

The Examiner believes that the abbreviation "HSC" means human stem cell and that the abbreviation "HPC" means human progenitor cells. "HSC" actually stands for hematopoietic stem cell, and "HPC" actually stands for hematopoietic progenitor cells. HPCs are simply cells from which various types of blood cells can arise. HPCs exist in many states of differentiation. For example, highly-differentiated HPCs can become only a limited number of types of blood cells. Less highly-differentiated HPCs can become any of a larger number of types of blood cells. HSCs are highly non-differentiated HPCs. Thus, all HSCs are HPCs, but not all HPCs are HSCs.

An important aspect of the invention is that the inventors have discovered that KDR^+ HPCs comprise HSCs that are able to repopulate hematopoietic tissues over the long term, and that an HPC population can be enriched for such long term-repopulating HSCs by selecting KDR^+ HPCs. The methods recited in the claims are directed to obtaining long-term repopulating HSCs. The HSCs are obtained from mixed populations containing many types of HPCs. For this reason, the claims recite methods of isolating HSCs by manipulating HPCs.

The Applicants have used the terms "HSC" and "HPC" in the clearest possible way to refer to particular cell populations and cell types. The final clause of claim 18 indicates that KDR^+ HPCs are enriched for long-term repopulating HSCs. The Examiner contends that it is unclear whether HSCs are isolated in a single step or in multiple steps. The Applicants reply that it does not matter whether the HSCs are isolated in one step, two steps, or a greater number of steps, so long as they are isolated from i) the human hematopoietic tissue recited in claim 18 and ii) KDR^- HPCs. The Applicants therefore respectfully contend that the meaning of each of claims 18-32, 39-44, and 75 is clear to the skilled artisan.

The Examiner also suggests that recitation of "the HSCs" in each of claims 24, 25, 27, 39, and 75 lacks antecedent basis because claim 18 recites only KDR^+ HPCs. As an initial matter, the Applicants note that none of claims 24, 27, 39, and 75 recites "the HSCs" and that the Examiner's concern should not apply to those claims. Claim 25 recites "the long-term repopulating HSCs." The final clause of claim 18 ("wherein the isolated KDR^+ HPCs are enriched for long-term repopulating HSCs") indicates the HSCs which are referred to in claim 25. The Applicants do not believe that any clarifying amendment of the rejected claims is necessary, but would consider any suggestion offered by the Examiner in this regard.

The Applicants believe that the foregoing issues were discussed with the Examiner during the telephone interview, and that the Examiner was satisfied that the claims, as amended, do not lack clarity.

For the foregoing reasons, the Applicants respectfully request reconsideration and withdrawal of the rejection of claims 18-32, 39-44, and 75 pursuant to 35 U.S.C. § 112, second paragraph.

Rejection of Claims Pursuant to 35 U.S.C. § 102

Each of claims 1-5, 18, 19, 21-27, 39, 51-53, 69 and 73 stands rejected pursuant to 35 U.S.C. § 102(a) or § 102(b) in view of one or more of Bhatia, Sutherland, Zanjani, Brandt, and Matthews. In the Examiner's view, the methods disclosed in these references would inherently result in purification of cells that express KDR on their surface.

Each of the pending claims recites use of "a reagent that specifically binds with KDR." None of the references discloses using a reagent that binds specifically with KDR. Neither does any of the cited references suggest using such a reagent. For this reason, the Applicants respectfully contend that none of the references cited by the Examiner anticipates the claimed methods. The Applicants believe that the Examiner's agreement with this contention was reached during the telephone interview. Reconsideration and withdrawal of the Examiner's rejection of the claims pursuant to 35 U.S.C. § 102(a) § 102(b) in view of one or more of Bhatia, Sutherland, Zanjani, Brandt, and Matthews are respectfully requested.

Summary

The Applicants submit that each rejection of the claims has been either overcome or is now inapplicable, and that each of claims 1-5, 7-11, 18-32, 51-53, 69, and 71-100 is now in condition for allowance. Reconsideration and allowance of each of these claims are respectfully requested at the earliest possible date.

If the Examiner believes that this application is not in condition for allowance for any reason, the Examiner is requested to telephone the Applicants' undersigned representative.

Respectfully submitted,

CESARE PESCHLE *ET AL.*

11 June 2002 (Date)

By: _____

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Enclosures
- Petition for Extension of Time
- Clean Copy of Replacement Specification Paragraphs
- Marked-Up Copy of Replacement Specification Paragraphs
- Clean Copy of Claims, As Amended
- Marked-Up Copy of Amended Claims
- Sequence Listing (paper and electronic copies and Statement to Support)